SOILS AND SEDIMENTS AS MATRICES FOR MICROBIAL GROWTH

D. B. NEDWELL AND T. R. G. GRAY

Department of Biology, University of Essex, Colchester, Essex CO4 3SQ, UK

This review will highlight some of the factors affecting microbial growth that are characteristic of soils and sediments. The bulk of the volume of a soil consists of finely divided, inorganic materials which do not act as substrates for metabolism but nevertheless have a profound effect on the liquids, inorganic and organic solutes, gases and microorganisms that occupy the pores between the particles. In sandy materials, only about 30% of the volume is pore space, although the pores are relatively large in diameter. In clays, 50% of the volume is pore space, although the pores are much smaller in diameter. These pores are filled with liquids and gases to varying degrees. Heterotrophic microbial growth will only be sustained in these matrices if there is a sufficient input of organic material. Peculiarities of the supply, distribution and utilization of this organic material will also be considered.

One of the major consequences of the abundance of solids is that movements of organisms, water, gases and substrates are much more restricted than in aqueous environments. This leads to the development of environmental heterogeneity and it is with this aspect that we start.

THE DEVELOPMENT OF HETEROGENEITY IN SOILS AND SEDIMENTS

Gross heterogeneity

Soils arise from the laying down of weathered particles formed from parent rock material (Foth, 1984). If the rate of erosion is less than the rate of particle formation, a soil will develop on top of the parent material. The processes which lead to the formation of a soil will vary in their duration and intensity, resulting in the formation of different soil types. The primary factors that affect these processes will be the nature of the parent rock material, the climate and the

types of macro- and microorganisms that become established. During the long process of soil formation, major climatic changes have occurred so that today's soils may reflect not only current factors and processes but those of preceding eras. Most soils have developed during the last hundred million years but in the temperate regions which have been subject to repeated glaciations over the last 1.5 million years the soils are relatively recent. However, even in these soils much of the boulder clay deposited by the glaciers contains previously weathered clays as well as newly weathered materials.

During the development of soils, the solid matrix is relatively stable although subject to periodic disturbance through the action of burrowing animals, root growth and cultivation. A consequence of stability is that vertical environmental gradients develop between the underlying parent material and the above-ground atmosphere. These gradients are caused by differences in the relative rates of production and consumption of organic matter at the surface and at depth in the soil, differences in the relative rates of production and removal by plant roots of inorganic materials, differences in the relative rates of production, consumption and diffusion of carbon dioxide, oxygen and other gases in the atmosphere and the soil, and the progressive chemical and physical interaction between the organic and inorganic solids and downward-percolating rain water (or water rising from the water table by capillary action). Over a period of time, interactions between these different gradients result in the formation of more or less distinct soil horizons, each providing fundamentally different environments for microbial growth. Fig. 1*a* and *b* show the variation in a number of important factors that would affect microbial growth in a podsol and an altosol, soil types from which are derived many of the present-day soils in Great Britain. The number of horizons in a soil profile changes with time. Surface horizons appear first during a few decades, the middle horizons next during the first 5000 years, and the deeper horizons over even longer periods (Fitzpatrick, 1971).

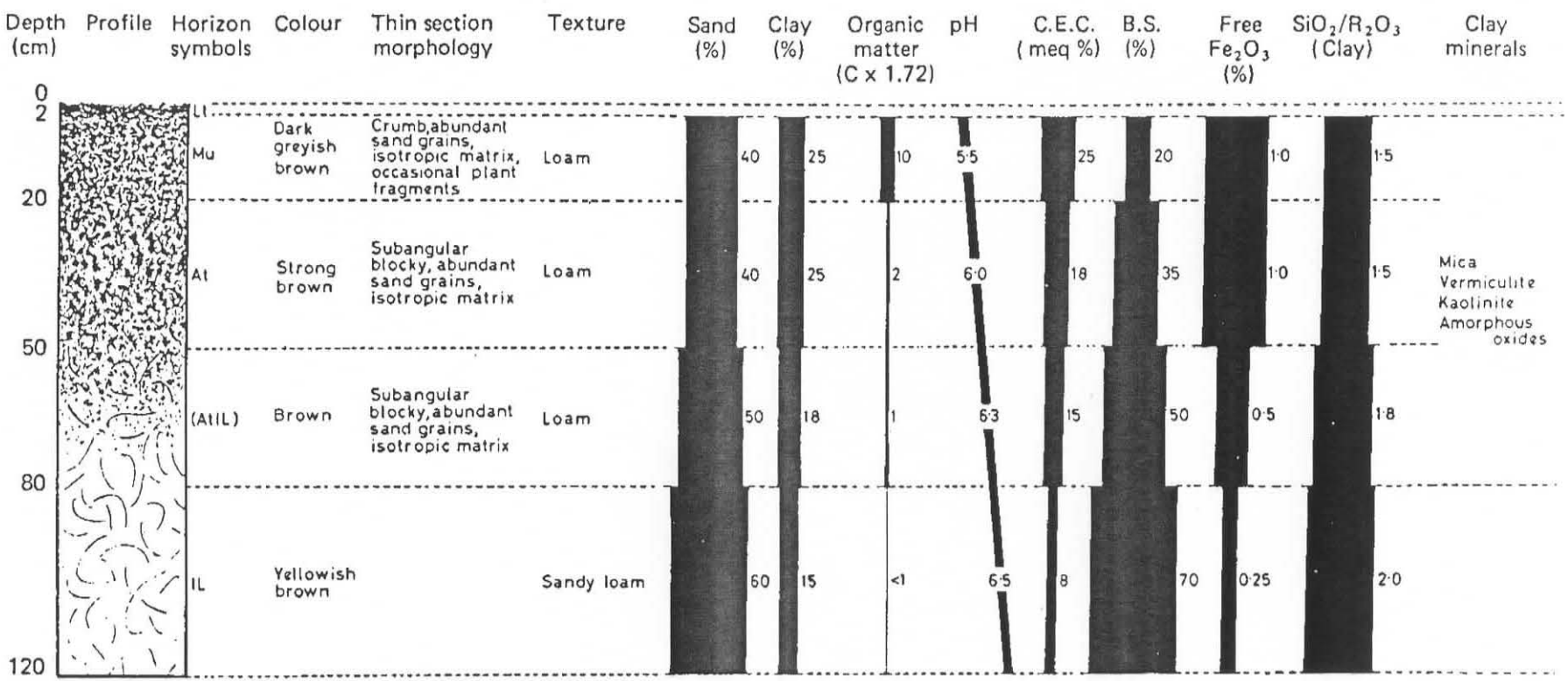
Erosion of terrestrial soils may cause movement of the particles through air or water to be deposited elsewhere as a sediment, e.g. as dunes or riverine, lacustrine, estuarine and marine deposits. Characteristically, these sediments are relatively homogeneous in respect of particle size at any one place, as the rate of sedimentation is dependent on particle size and the depositional environment. Small particles which remain in suspension even at low current velocities are transported for greater distances, hence the occurrence of finer

muds on the upper reaches of tidal marshes. However, processes leading to erosion and transport will vary in intensity over periods of time so that layers of different-sized particles may be deposited on top of one another. In physically high-energy environments, surface materials may be subject to turbulence so that solids are constantly resuspended and deposited, together with any microorganisms that are in them. Away from the continental shelf, oceanic sediments are dominated by particulate material, principally carbonates and silicates, which are of biogenic rather than terrestrial origin and derived from planktonic microorganisms.

Typically, aquatic sediments have a surface aerobic layer of variable depth, below which the sedimentary environment is anaerobic (see also p. 35). The depth of the aerobic layer depends upon a balance between the rates of oxygen removal by the sedimentary microflora and diffusion of oxygen into the sediment from the overlying water. Factors which stimulate respiration, such as increased temperature and increased supplies of organic carbon, decrease the depth of the aerobic layer. Heterogeneity within sediments is associated principally with the establishment of vertical concentration gradients of solutes. These may be produced through removal of electron acceptors, e.g. oxygen, nitrate and sulphate, from the sediment during microbial respiration, resulting in solutes diffusing into the sediment from the overlying water. The products of detrital mineralisation, such as phosphate and ammonium ions, are usually higher in concentration in the sediment than the overlying water and so they diffuse upwards. Bottom sediments are thus both important sinks and sources of inorganic nutrients for the plankton.

The amount of organic material reaching aquatic bottom sediments is a function of the depth of the overlying water column. Microbial decay of organic material as it settles through the water column progressively reduces the total amount of surviving organic material with increased depth of water (Fig. 2), and also changes its quality as the more labile, degradable organic components are removed. In the deep ocean only a small fraction of the most refractory organic components of the detritus, initially derived from primary production in the euphotic zone, may survive to reach the sediment/water interface, and this is reflected in the trend of reduction of the organic carbon content of sediments as water depth increases (Fig. 3). The effect upon microbial activity is illustrated by the similar trend of decrease of oxygen uptake rates by sediments with increased water depth (Fig. 4), reflecting diminished respiratory

a


Depth (cm)
Profile
Horizon symbols
Colour
Thin section morphology
Texture
Sand (%)
Clay (%)
Organic matter (C x 1.72)
pH
C.E.C. (meq %)
B.S. (%)
Free Fe_2O_3 (%)
SiO_2/R_2O_3 (Clay)
Clay minerals
0
2
20
50
80
120
Lt
Mu
Dark greyish brown
Crumb,abundant sand grains, isotropic matrix, occasional plant fragments
Loam
40
25
10
5·5
25
20
1·0
1·5
At
Strong brown
Subangular blocky, abundant sand grains, isotropic matrix
Loam
40
25
2
6·0
18
35
1·0
1·5
Mica
Vermiculite
Kaolinite
Amorphous oxides
(AtIL)
Brown
Subangular blocky,abundant sand grains, isotropic matrix
Loam
50
18
1
6·3
15
50
0·5
1·8
IL
Yellowish brown
Sandy loam
60
15
<1
6·5
8
70
0·25
2·0

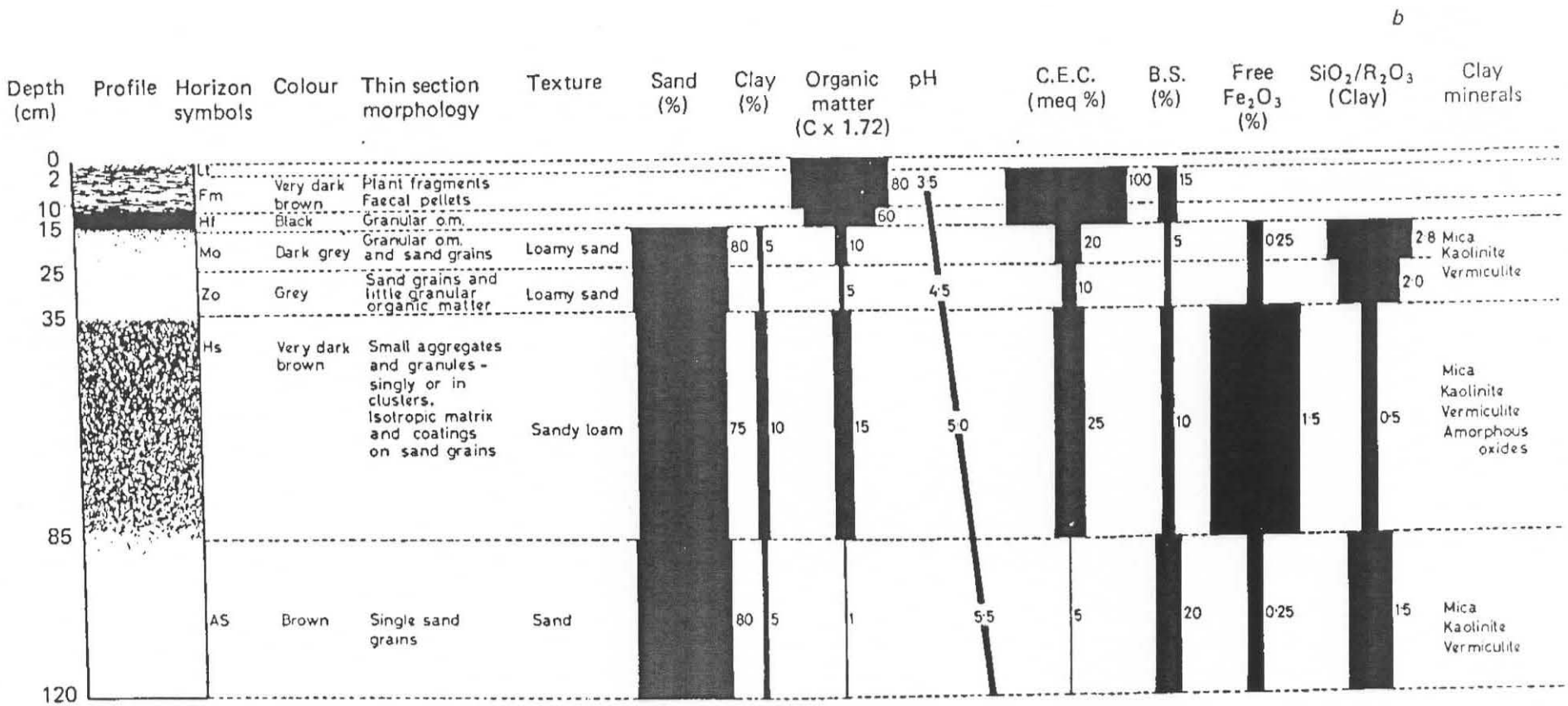


Fig. 1. A comparison of environmental data for the horizons of two soil profiles: (a) an altosol (brown earth), with Lt (litter), Mu, (mullon or A_1), At (alton or A_2) and IL (underlying material) horizons; and (b) a podsol, with Lt (litter), Fm (fermenton or F), Hf (humifon or H), Mo (modon or A_1), Zo (zolon or A_2), Hs (husequon or B_{hfe}) and AS (underlying material) horizons. G.E.C. = Cation Exchange Capacity expressed as meq per 100 g of soil of the <2 mm size fraction; B.S. in Base Saturation expressed as a % of G.E.C.; R = Fe + Al; o.m. = organic matter. (After Fitzpatrick (1971), with permission from Longmans).

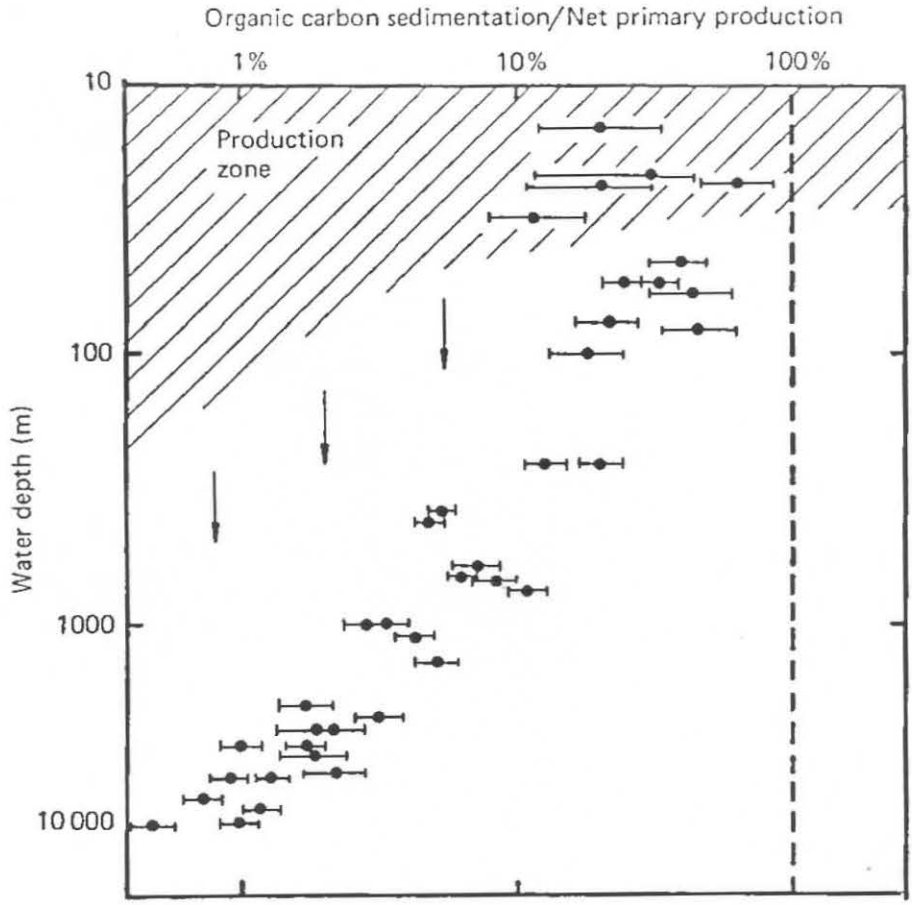


Fig. 2. Amounts of residual organic matter remaining with depth in the water column after settlement from the euphotic zone. Reprinted with permission from *Nature*, **288**, 262–3,

activity of the microbial community as availability of organic electron donors decreases with increased water depth.

In historical times, soil and sediment development have been greatly influenced by man's activities. Man has altered natural vegetation patterns, producing large areas of heathland, savanna and prairie and thus modifying the soil, as well as improving soils through reclamation from the sea and devastating others through over-exploitation.

This degree of complexity at the gross level ensures that the data from laboratory model systems are of limited value. The comparison of the additions, losses, translocations and transformations of materials in a field soil with those in a column containing an artificial matrix through which a medium is flowing shows why this is so (Fig. 5*a* and *b*, adapted from Foth (1984)). Laboratory models are extremely useful tools for investigating individual, underlying processes in soil, but less useful for understanding how these different processes interact. They are also useful for studying short-term processes which

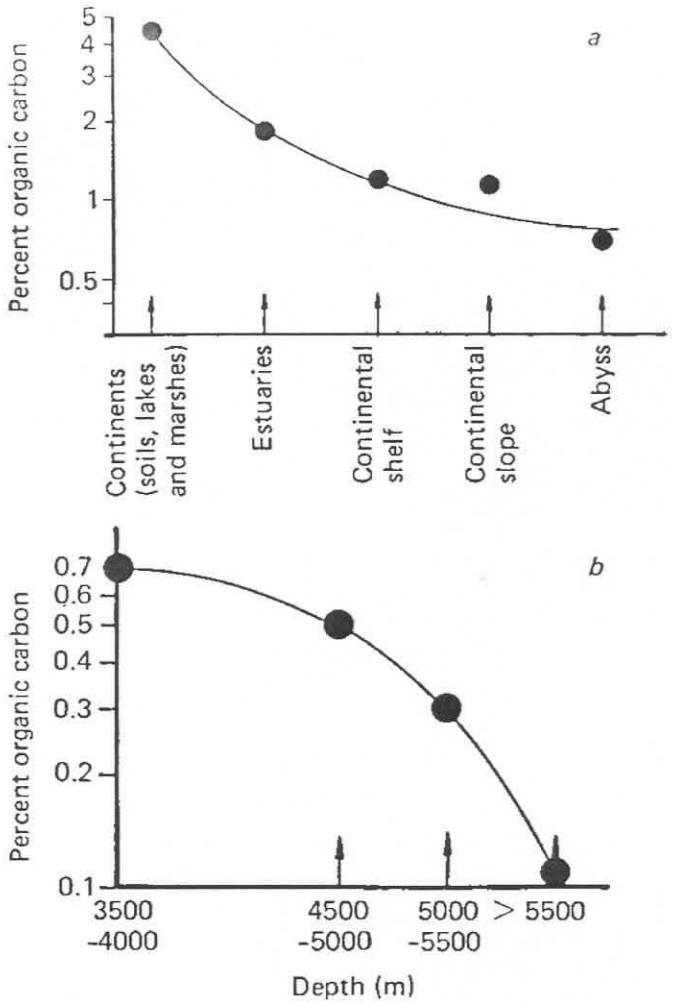


Fig. 3. Average organic carbon contents of sediments: (a) in major types of sedimentary environments; (b) in marine abyssal sediments. The depth axis is not to scale. (Redrawn from Vigneaux *et al.*, 1980.)

may or may not be related to long-term changes taking place in the natural environment.

Heterogeneity of microhabitats

In space

Although soil and sediment horizons each have characteristic properties, considerable variation in the environmental factors that affect microbial growth occurs from point to point. For heterotrophic bacteria, it is the distribution of their organic substrates which primarily determines the occurrence of pockets of activity, sometimes referred to as microhabitats. The majority of the organic substrates entering soil are either insoluble or are soluble but packaged in cells and tissues with insoluble boundaries. Before decomposition takes place, therefore, there will be limited dispersion of the substrate within

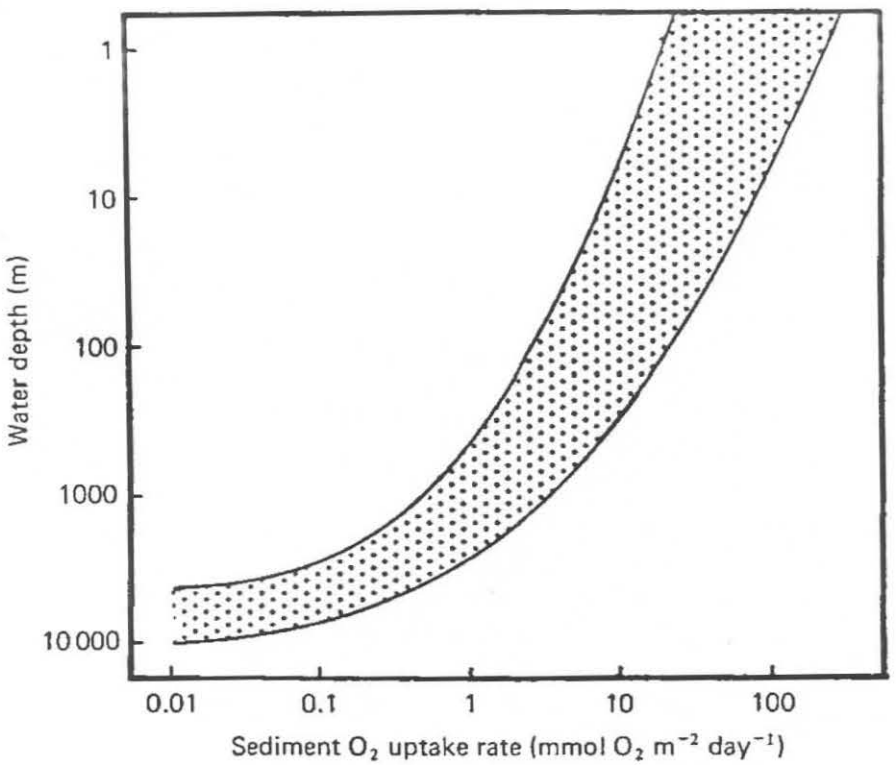


Fig. 4. Uptake of oxygen by microorganisms in marine bottom sediments in relation to depth of overlying water. (Redrawn from Jørgensen (1983), with permission from SCOPE.)

the inorganic soil matrix, and litter material often spends a considerable period of time at the surface in the O horizons where much comminution and decomposition takes place. Nevertheless, some soluble materials will diffuse into the soil water and be leached out of the surface horizons. Also, animals may transport and distribute larger pieces of organic material unevenly within the soil matrix.

Organic matter is also produced within the soil by plant roots. In 1 ha of a 50-year-old pine forest, the soil to a depth of 200 cm may contain about 4 tonnes of living roots less than 0.3 mm in diameter, about 50% of which are replaced each year. This biomass of roots may represent 50% of the mass of an intact tree (over 90% in the case of some grasses). At the soil–root boundary, microorganisms can colonize the surfaces of living cells and root hairs as well as crushed and partly disorganised cells. They can also penetrate and utilise the contents of living cells, exploit the mucigel and sloughed off cells, and use the soluble exudates that diffuse out from the younger parts of roots. Living roots raise the pH of the soil by as much as 1 pH unit for a few millimetres around the root and set up water and ion gradients as well.

The extent of these gradients, as well as gradients of soluble breakdown products of organic matter, will be affected not only by the

diffusion coefficients of the different substances but also by the degree of discontinuity of the water films in the soil pores and the amount of downward mass flow of water. Solute diffusion decreases approximately linearly with volumetric water content so that it will be restricted at low matric potentials, potentials that will also restrict bacterial movement (Griffin, 1972) (see below). All these factors will be influenced by variations in the textural composition of the soil and rates of loss of water through evaporation or drainage. When the pore spaces between the solid particles are water-filled, then the pattern of decomposition of the substrate will change, with anaerobic processes predominating where rates of oxygen consumption exceed rates of supply of oxygen by diffusion or percolation. Thus, surrounding a solid substrate will be a volume of soil, changing in diameter with time, into which miocroorganisms can grow outwards to exploit dissolved nutrients and spread to other substrate pockets. Organisms with a limited ability to spread and disperse will be at a disadvantage, for the amount of substrate present in any one microhabitat will be small and readily exhaustible. Their only alternative strategy will be to reduce their metabolic rate by forming resting stages and to wait for fresh substrate to arrive or to be carried to substrate by burrowing animals.

An uneven distribution of substrate would be expected to influence the morphological types of microorganism that could grow well. In those types of aquatic environment in which the majority of substrate is soluble, small unicellular organisms with large surface:volume ratios are favoured. However, in soils and sediments only some of the substrate is soluble and hence occurs in the water films in the pore spaces. The remaining solid substrates may be adsorbed onto inorganic particles, or trapped inside clay lattices, or they may exist as large discrete particles. The decomposition of such solid substrates presents several interesting features:

1. they are usually small in quantity at any one point, so that rapid microbial growth will cease quickly when the growth-limiting nutrient is exhausted;
2. for rapid exploitation, penetration of the substrate is desirable; for slow exploitation, gradual erosion of the surface is sufficient;
3. individual substrate particles may be separated by considerable distances (in microbial terms), although individual substrate particles may be ingested or moved in other ways through animal activity;

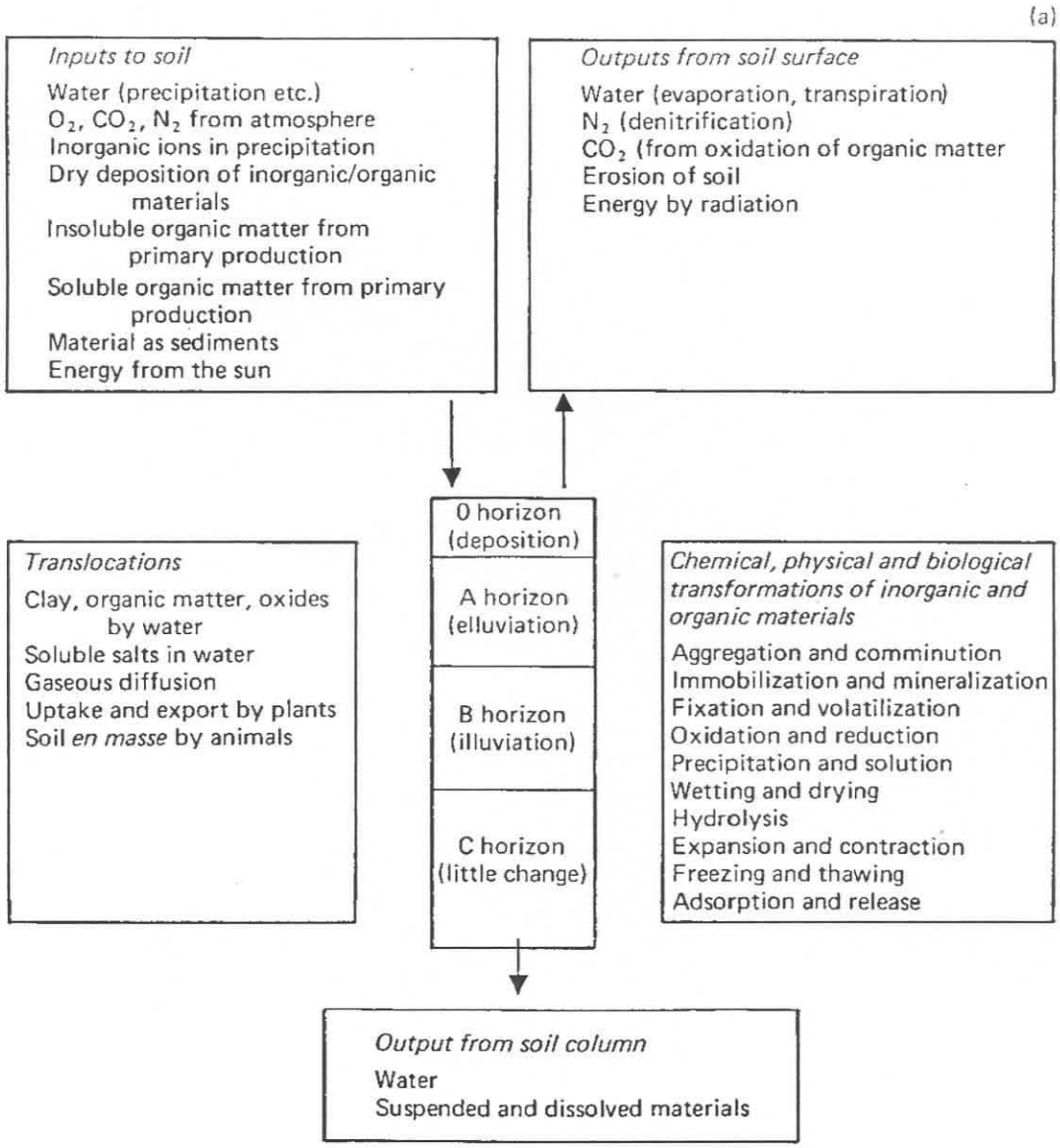
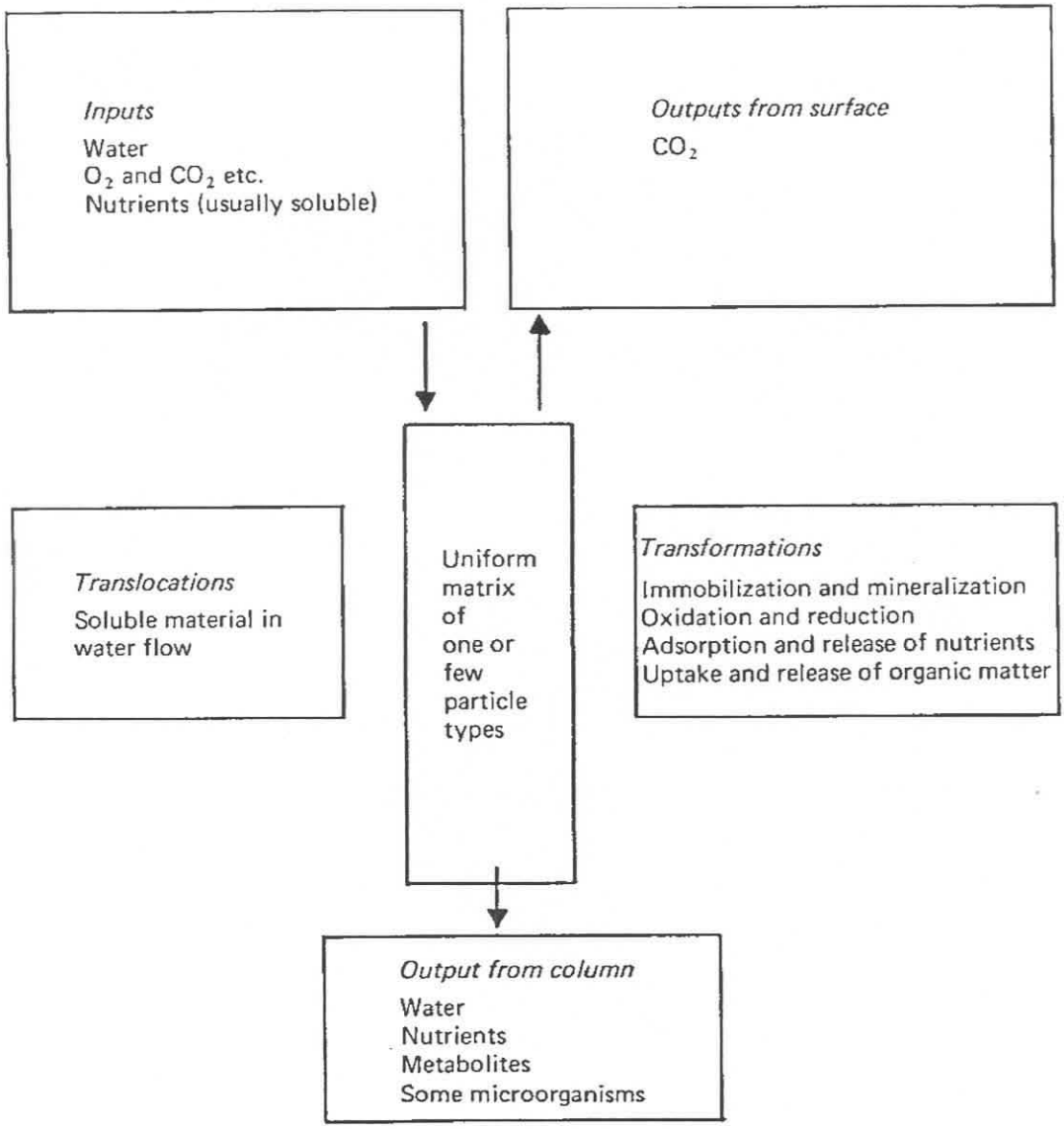


Fig. 5. A comparison of inputs, outputs, translocations and transformations taking place in (a) a soil (modified from Foth, 1984) and (b) a glass column supplied with a nutrient medium and containing a pure culture of a heterotrophic bacterium. Note that the time scale for the soil system is a long one, during which many environmental changes and fluctuations will occur. The time scale for a model system is generally short and environmental conditions are kept constant.

4. in soils with a clay content greater than 12–14%, aggregation of particles to form crumbs will occur; aggregate formation may entrap both substrate and microorganism, effectively isolating them from other parts of the soil;
5. humified materials are often deposited in particular horizons in a soil profile; organisms exploiting these substrates may need to adhere to the soil particles to avoid being separated and washed out of the soil.

(b)



Thus, organisms inhabiting soil are often found to have developed one or more of the following characteristics: filamentous/mycelial/cord habit or small colonies of unicells; resistant wall structures; dormant propagules or shut-down cells; adhesive mechanisms; dimorphic or pleomorphic potential. Gray & Williams (1971*a*) proposed a series of growth patterns of microorganisms in soil which incorporated this diversity. These seven patterns were: (i) non-migratory unicells, (ii) migratory unicells, (iii) plasmodia, (iv) substrate-restricted hyphae, (v) locally spreading hyphae, (vi) mycelial strands/rhizomorphs, and (vii) diffuse spreading hyphae. These concepts have been refined and expanded by Cooke & Rayner (1984; see also this volume), partly in the realization of the pleomorphic

potential of many organisms. Dowson, Rayner & Boddy (1986) have shown that wood blocks placed about 5 cm from an inoculated wood block cause marked changes in the form and growth characteristics of the mycelial network of two basidiomycetes, *Hypholoma fasciculare* and *Phanaerochaete velutina*. Initially, sparse exploratory mycelial systems were produced from the inoculated block. The direction of growth was changed toward the uninoculated block and further radial extension ceased following contact with it. Regression of the mycelial front not in contact with the block followed and at the same time fan-shaped systems of effuse mycelium spread over the uninoculated block.

It is clear from these experiments that fungi have the potential to change their growth pattern in response to the presence of substrates. The most dramatic change that can take place is in those dimorphic forms capable of existing as yeasts or mycelium. It is generally considered that yeasts are at a disadvantage in soils and sediments, as they are incapable of penetrating solids and have limited ability to disperse themselves by growth or spore production. They are rarely isolated from soils, except where they have found their way into the soil from surfaces of living leaves and fruit where they exploit exuded carbohydrates.

However, other unicellular colonial organisms such as bacteria do not seem to be at such a serious disadvantage for they can produce a sizeable biomass, e.g. Gray, Hissett & Duxbury (1974). But where are these other unicellular forms found? The majority of them probably occur on plant surfaces. In this case the surfaces are those of roots (Foster & Rovira, 1976) or hyphae (Siala & Gray, 1974; Fradkin & Patrick, 1982), where they utilise the nitrogen-rich, soluble exudates or metabolic byproducts of fungal decomposition of insoluble polymers. Those bacteria that are found on non-living, inorganic, solid particles occur in very small colonies, the majority of them on particles coated with iron and humified materials (Siala, Hill & Gray, 1974). They may well obtain many of their nutrients from water percolating through the soil. However, Griffin & Luard (1979) have pointed out that if a cell is on a solid surface, it and its progeny will be trapped by surface tension at one site unless the water film is at least as thick as the diameter of the cell. Furthermore, for movement to occur, the neighbouring pore system must be water-filled and the pore necks big enough to allow passage of the cell. Thus, if the matric potential of a soil is -147 kPa or less, pore necks of 1 μm radius will be air-filled and restrict bacterial movement.

Movement of larger yeasts or fungal zoospores (radius 10 μm) would be prevented when the matric potential of the soil reached −14.7 kPa. In soils where pore neck sizes are highly variable, the probability of a continuous water-filled pathway existing is much reduced, so that even a matric potential of −100 kPa, equivalent approximately to a water content of 20% in a loam soil and 50% in a clay soil, will have a marked effect on bacteria and an even bigger effect on yeasts and zoospores. The activity of unicellular organisms is thus limited by exhaustion of nutrients following a decline in the matric potential. It is not surprising, therefore, that Chuang & Ko (1981) found that there is an inverse linear relation between propagule volume and abundance of microorganisms in soils (on a logarithmic basis), though this will be due partly to the impossibility of sustaining large numbers of large propagules on the energy available. Lundgren (1984) has also found that most soil bacteria are smaller than 1 μm and that 55% are smaller than 0.5 μm. Soil protozoans would also be limited by the number of available pore spaces. Darbyshire, Robertson & Mackie (1985) have measured the distribution of different-sized pores above 3 μm in diameter in agricultural soils which would allow the passage of protozoans, but data on the water status of these pores are not available. Filamentous organisms behave differently for they are active at lower values of matric potential. Their growth is prevented not indirectly by nutrient exhaustion but more directly by matric potential and water availability. It is noteworthy that matric potentials can be much lower than solute potentials in nature and that in some dry soils survival of xerotolerant forms only can be expected (Lanyi, 1979).

Despite the presence of pores through which oxygen can diffuse rapidly, anaerobic environments also occur in soil, especially when there is a high clay content and a tendency to produce aggregates. The finer pores inside the aggregates remain water-filled, even at quite low matric potentials, and Greenwood (1961) has estimated that soil aggregates with a diameter greater than approximately 3 mm (depending upon temperature, organic matter content and matrix texture) will have anaerobic centres. Larger crumbs will thus have an anaerobic core surrounded by an aerobic shell. However, microbial activity in the core may be negligible, as substrates which are rapidly exhausted there will not be replaced (Allison, 1968).

In some parts of the soil profile, notably the litter layers, organic substrates and aerobic environments are not discontinuous. In these habitats, spreading hyphae of fairy-ring type fungi are extremely

common where growth, albeit slow, is reminiscent of that occurring on agar plates (Cooke & Rayner, 1984). However, in these environments, fungi may still not be distributed uniformly, even if they are indiscriminate in their choice of substrate. An excellent example of such a fungus is *Mycena galopus*, which has the potential to attack all the major constituents of plant litter and is known to colonize a wide variety of broad-leaved and coniferous litter types (Frankland, 1984). It grows in close proximity with other basidiomycetes such as *Marasmius, Collybia* and *Cystoderma.* In laboratory experiments, *Mycena galopus* was outcompeted by *Marasmius androsaceus* when growing on sterile spruce litter but, in the field, these two fungi were separated vertically, with the mean depth of origin of the fruit bodies of the former being 6 mm and of the latter, 1 mm (Fig. 6). It has been suggested by Newell (1984) that this distribution is

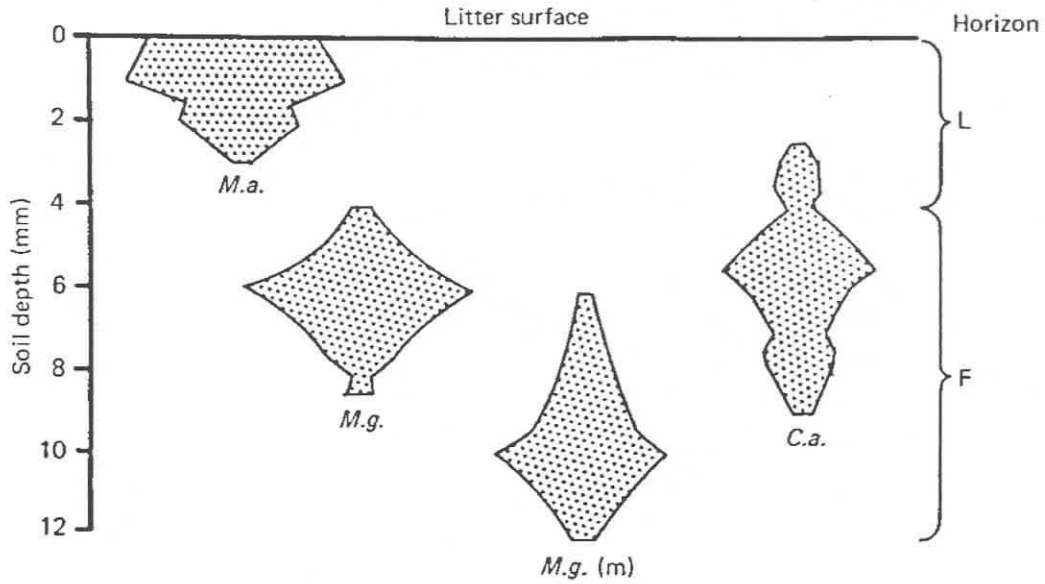


Fig. 6. The fruiting depths of *Marasmius androsaceus* (*M.a.*), *Mycena galopus* (*M.g.*) and *Cystoderma amianthinum* (*C.a.*) in a *Picea sitchensis* plantation. *M.g.* (m) = mixed species clumps of *M. galopus* with *M. androsaceus.* The width of the kite bars at any depth is proportional to the percentage number of basidiocarps originating at that depth. (After Frankland (1984), with permission of the British Mycological Society.)

caused partly by the collembolan mite *Onychiurus latus* which grazes differentially on these fungi, preferring *M. androsaceus* mycelium. These collembolans are most abundant at a depth of about 6 mm. Dix (1984) has also shown that *Mycena galopus* is less tolerant than *Marasmius androsaceus* of low water potentials, and thus the observed field distribution may be due to a combination of physical and biological factors. Frankland (1984) has also shown that *Mycena galopus* is not distributed at random horizontally over the forest floor: surprisingly, its position is related to the position of trees even

though it is not mycorrhizal. Further studies on the distribution of this fungus may be assisted by the development of specific fluorescent antibodies that will enable its mycelium to be identified *in situ* in the absence of fruit bodies; such antisera are now being developed (Frankland *et al.*, 1981; Chard, Gray & Frankland, 1983, 1985*a,b*).

In waterlogged sediments the picture is very different, for here the environment is often anaerobic, fungi are absent and bacteria play a much greater role in decomposition. The diffusion coefficient of oxygen in water is about 10^4 times lower than in air and the concentration of oxygen in water is also low, there being only 0.28 mmoles l^{-1} at 20° C in freshwater and 0.23 mmoles l^{-1} at 20° C in seawater. Thus, it is easy to understand why oxygen-consuming, waterlogged systems become anaerobic below the surface. The depth of the surface aerobic layer is a function of several different factors. Increases in available organic matter and temperature enhance respiratory removal of oxygen and thus diminish the aerobic layer; increases in sediment particle size and porosity enhance transport rates and extend the depth of the aerobic layer. However, sulphate reduction can occur in anaerobic microenvironments, even within the generally aerobic surface layers of marine sediments, possibly associated with localised organically rich copepod faecal pellets where oxygen is consumed rapidly (Jørgensen, 1977). In a highly organic sediment with a restricted depth of aerobic layer, a significant proportion of the organic material deposited on the surface may pass through the aerobic zone and be mineralised anaerobically. In marine sediments, the major electron acceptor after oxygen removal is sulphate, which is present at approximately 20 mM concentration in seawater, compared to oxygen at approximately 0.23 mM concentration. Jørgensen (1982) has emphasised the importance of sulphate reduction in the mineralisation of organic material in those shallow-water marine sediments which have a large input of organic matter and a restricted aerobic layer. Approximately 50% of the total mineralisation of organic carbon is brought about by sulphate-reducing bacteria in these environments. By contrast, deep oceanic sediments have a low organic input, are at low temperature and have extensive aerobic layers in which the majority of organic carbon is mineralised aerobically. Sulphate reduction is therefore less important in these deep-water marine environments because of the extensive aerobic layer and it is of little quantitative significance in freshwater sediments as freshwater contains little sulphate. Nitrate reduction and carbon dioxide reduction (methane formation) are

the most important respiratory processes in anaerobic freshwater sediments (Jones & Simon, 1980).

In time

Microenvironments are not only spatially heterogeneous but also heterogeneous with time. Bosatta & Berendse (1984) have reviewed the ways in which the nutrient composition of substrates can change. Thus, during litter decomposition a phase of nitrogen accumulation is succeeded by a period of nitrogen release, a change which probably also occurs with other mineral nutrients (Swift, Heal & Anderson, 1979). The change from one phase to the other depends upon a change in the carbon:element ratio of the litter. Bosatta & Berendse (1984) point out that carbon mineralisation is negatively correlated with nitrogen mineralisation in both laboratory and field experiments. Thus the common idea that the priming effect of nitrogen additions on soil nitrogen mineralisation is due to enhanced microbial activity may not be true. A qualitatively different response to the same perturbation can be expected depending upon whether the system is carbon- or nitrogen-deficient. In unperturbed systems, Bosatta & Staaf (1982) showed that the retention and release of nitrogen was regulated by decomposition rate and initial nitrogen concentration of the litter. Increased decomposition rates reduced the rates of nitrogen release per unit of carbon mineralised. Litters with a low initial nitrogen concentration immobilised more nitrogen but over a shorter period of time. Thus, in all litter types, carbon:nitrogen ratios converge as decomposition proceeds.

More recently, Bosatta & Agren (1985) have proposed a theory for the microbial decomposition of heterogeneous substrates in soil. They point out that decomposing organic matter is heterogeneous both because of its original composition and because of new compounds produced during decomposition and humification. Both the carbon left in the substrate and that returned by the microbes can be thought of as being of lower quality than that which was present originally, i.e. more refractory. This, as decomposition proceeds the substrate becomes progressively poorer in quality. Quantity and quality of soil organic matter are the result, therefore, of interactions between many processes with time constants ranging from seconds to thousands of years.

The patterns and rates of decomposition could be described, according to Bosatta & Agren (1985), in terms of a few critical functions of the microbial population (mean concentration of carbon

in the microbial biomass, mean concentration of nitrogen in the microbial biomass, efficiency of carbon utilisation, the rate of substrate utilisation), the quality of the carbon resource, and the initial quality and nitrogen:carbon ratio of the litter. The most important functions were the microbial efficiency and the rate of substrate utilisation. Bosatta & Agren pointed out that if the degradation in substrate quality were more rapid than that in substrate quantity, then eventually a finite amount of undegradable material would remain undecomposed. For *all* the material to be degraded, the fall in quantity would need to be more rapid than that in quality which could be achieved by decreasing microbial efficiency as quality decreased. Application of theoretical models to experimental data obtained by McClaugherty *et al* (1985) for the decomposition of aspen, hemlock, white maple, sugar maple, red maple and white pine leaves over 2 years suggested that the efficiency of utilisation of litter varied from 0.19 (white pine) to 0.367 (aspen). The assumption that the efficiencies remained constant during decomposition was shown to fit the data, except for the final observations in the time series. These coincided with a second period of nitrogen mineralisation and a shift in extracellular enzyme activity. McClaugherty *et al.* ascribed this to a change from one microbial community to another with a different and lower efficiency. If this interpretation turns out to be correct, then these latter organisms may be regarded as truly autochthonous (Winogradsky, 1924).

Soils and some sediments may be subject also to seasonal changes. Soil is a poor conductor of heat so that large temperature changes in the atmosphere may not change soil conditions much below the surface layers except in extreme environments. Nevertheless, some other major seasonal changes in the environment will occur in subsurface soil, mostly related to changes in the moisture content and consequent degree of aeration. The buffering effect of surface soils on sub-surface horizons is illustrated by data obtained from soils during forest fires, where surface temperatures of 850° C can be reached while soil at about 4 cm depth remains at 16° C (Hofmann, 1917). As soils are often dark in colour, direct insolation can also cause surface soils to increase in temperature well above air temperature. Antarctic fellfield soils can experience diurnal temperature changes of 40° C, although these effects are damped out with increasing depth (Fig. 7) and only longer-term seasonal temperature cycles are observed in deeper layers.

In intertidal sediments which are exposed to air, seasonal, diurnal

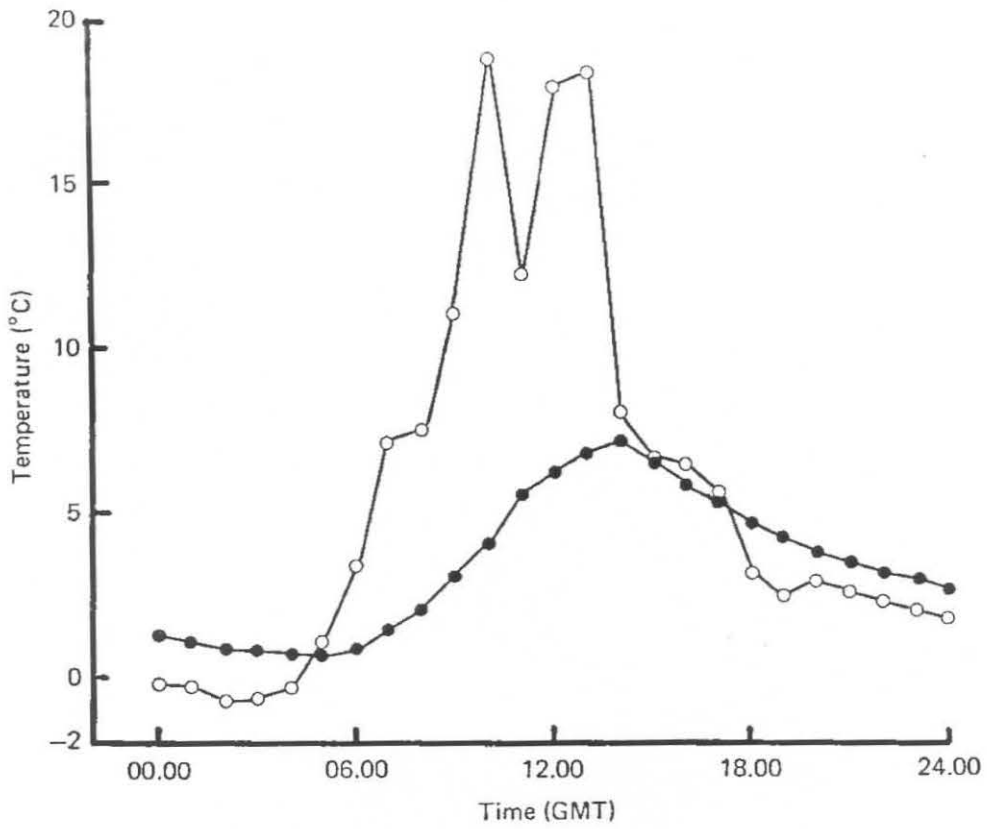


Fig. 7. Diurnal temperature changes at the surface (○) and at 10 cm (●) depth in an Antarctic fellfield soil during summer. (Unpublished data from D. W. H. Walton, British Antarctic Survey, Cambridge.)

and tidal cyclic changes occur and temperatures about 10° C above air temperature have been recorded in such surface muds in summer in Great Britain. However, water has a large thermal capacity, so that temperature changes in permanently submerged aquatic sediments are damped relative to those in air and these sediments are only affected significantly by seasonal changes: even these are small in deep oceanic environments. Other indirect seasonal changes can occur in deep sediments, however, because of the settling of organic detritus formed periodically in the euphotic zone. The microbial communities in different sediments may be influenced, therefore, by very different environmental regimes. When short-term variations, e.g. diurnal and tidal cycles, occur, speed of physiological response by a species to environmental change may be more important than its steady-state physiological competitiveness. Where only long-term variations occur, the reverse may be true.

Heterogeneity at the molecular level

The surface chemistry of soils has been reviewed extensively by Sposito (1984). Some of the solids present in soil exhibit considerable

surface activity, notably clays and humic materials. Since clays are, by definition, finely divided particles, the surface area they possess is enormous. In a cubic metre of clay, each particle having a diameter of 2 μm, the total surface area would be about $3 \times 10^6 m^2$. On this surface, in contact with the soil water, are many chemically reactive units separated by fixed distances, interacting with one another as well as with molecules in the water film. The principal reactive units on clay surfaces are siloxane ditrigonal cavities, inorganic hydroxyl groups and a variety of organic groups in humic substances which are themselves complexed with clay.

The siloxane ditrigonal cavity (diameter 0.26 nm) is formed by six corner-sharing silica tetrahedra and is thus bordered by six oxygen atoms, each with a set of lone pairs of electron-orbitals. If the clay has not undergone isomorphous replacement in the underlying layer of the molecule, these cavities act as electron donors and form unstable complexes only with neutral dipolar molecules such as water. However, if isomorphous replacement of trivalent aluminium with divalent iron or magnesium has occurred, e.g. as in montmorillonite, an excess negative charge is generated which enables cations to be complexed. Isomorphous replacement of tetravalent silicon with trivalent aluminium, e.g. as in vermiculite, also generates an excess charge but as it is distributed over fewer surface oxygen atoms than in the previous example, even stronger complexes with cations and dipolar molecules occur. Inorganic hydroxyl groups are the most abundant and reactive groups but their properties are varied, depending upon the atoms with which they are coordinated. They are capable of complexing hydroxide anions, hydrogen ions, oxyanions such as HPO_4^{2-}, and metal cations.

Organic compounds in the soil solution can be bound to clay particles and themselves then bind protons. The mechanisms underlying the adsorption of soluble organic compounds to clay include cation exchange, protonation, anion exchange, water bridging, cation bridging, ligand exchange, hydrogen bonding and van der Waal's interactions (Mortland, 1970; Greenland, 1971). Generally, the quantity of dissolved organic material adsorbed decreases as the pH increases above 4.0 (Theng, 1979). Humic materials have a number of surface functional groups, including carboxyl, carbonyl, amino, imidazole, phenylhydroxyl, sulphydryl and sulphonic groups. In well oxidised organic matter, the carbonyl, carboxyl and phenylhydroxyl groups are the most significant (Stevenson, 1982), binding protons with increasing degrees of relative stability. The variability

of the nature and position of the functional groups on the surface ensures that different interactions occur between the groups which, in turn, affect the stability of proton complexes.

The idea that enzymes may be immobilised by humic materials has been explored by Nannipieri, Ceccanti, Cervelli & Sequi (1978) and developed by Burns (1983). Recently, Serban & Nissenbaum (1986) suggested, on the basis of laboratory experiments, that extracellular enzymes such as peroxidase and catalase in the soil solution could become incorporated in the rigid, three-dimensional, macromolecular matrix of humic acid and that this rendered them resistant to decomposition by pronase and thermal denaturation but did not impair their activity. They suggested that humus-immobilised enzymes seemed to retain their activity more consistently than those bound to clay and might be responsible for the persistence of peroxidase-like activity in sediments 7 million years old.

The reactive groups at the soil surface all have profound effects on the water and its solutes surrounding the solid particles. Water is adsorbed and its structure altered in thin films. There is a more rigid configuration of water molecules interacting with fixed reactive groups on the clay surfaces and with cations in the water. These effects will be felt through several layers of water molecules so that adsorbed water extends for up to 3 nm around kaolinite, 5 nm around vermiculites and perhaps up to 10 nm around montmorillonite particles (Sposito, 1984). Adsorbed water has different solvent properties to bulk liquid away from the surface. There will be enhancement of complex formation between dissolved materials and between exchangeable cations and the siloxane ditrigonal cavity, thus retarding the development of the double diffuse layer. Acidity will also increase.

Microorganisms also possess fixed reactive groups on their surfaces, including COO^- and PO_4^{3-} groups (Rogers, 1979). The degree of attraction between particle and bacterium therefore depends upon a balance between electrostatic repulsion, electrostatic attraction and van der Waal's attractive forces. Marshall (1976) has shown that there are two distances of separation between surfaces at which net attraction will occur and that for adsorption to take place, the repulsion barrier between these two distances must be overcome. The degree of repulsion decreases with decreasing radius of curvature of the particles and increasing electrolyte concentration. Thus, as soils dry out and electrolyte concentration increases, the chance of adsorption will increase, so that bacteria, because of their

relatively large size, could become coated with clay particles or become attached to larger humus-coated sand grains. Stabilisation of such an attachment may require the formation of stronger bonds, through the production of fimbriae, lipopolysaccharides and peptidoglycan in the cell wall or extracellular polymers and capsules which are often polysaccharides (Fletcher & Marshall, 1982).

This high degree of variability and reactivity in the matrix of soils and sediments thus ensures that all the components involved in metabolism (water, anions, cations, hydrogen ions, organic matter, enzymes, metabolites and cells) will be distributed non-uniformly in relation to particle surfaces. Furthermore, the environmental conditions surrounding cells will be quite different from those occurring in 'liquid water' (Stillinger, 1980). Unfortunately, non-destructive methods of measuring these environmental factors are not available and so the precise conditions under which much decomposition takes place remain a matter for conjecture.

The potential complexity of substrate–clay–cell interactions has been underlined by Marshman & Marshall (1981*a*) who studied the growth of bacteria on pure proteins adsorbed on clay minerals. Their results were best explained by assuming that protein was bound at two sites; at one, the protein was available to bacteria, at the other it was not. These sites did not appear to coincide with internal and external lattice surfaces. In a second paper, Marshman & Marshall (1981*b*) suggested that clays such as montmorillonite could affect microbial growth through interactions with the organisms, their substrates, individual enzymes and growth factors. This increases markedly the complexity of the flow of nutrients to organisms. Dashman & Stotzky (1986) have also shown that complexes between different amino acids and peptides and montmorillonite are differentially available to microorganisms. The affinities of permeases for different amino acids are some 100–10 000 times greater than the affinities of clays for the same amino acids, so these are utilised readily; information on peptides was lacking. The differences in affinity of the permeases for different amino acids may account for the differences in the extent of their utilisation. They also postulated that the yield of energy from the intracellular metabolism of some amino acids might be less than the energy required to remove the substrate from clay and transport it into the cell. Thus cysteine was not utilised when bound to montmorillonite or kaolinite whereas proline and arginine were. The requirement for energy to be expended to remove materials from clay and humus may thus be an important consider-

ation when determining the relative amounts of energy available for growth and maintenance.

INPUT AND METABOLISM OF ORGANIC MATTER

As residual organic material sinks through the water column, it becomes increasingly refractory as it is depleted in nitrogen and phosphorus relative to carbon, the C:N:P ratio changing from approximately that of algae (106:16:1) near the surface to 106:3.5:0.11 deeper in the water column (Suess & Müller, 1980). In terrestrial systems, higher plant debris, with a relatively large component of refractory structural polymers such as lignin, cellulose and hemicelluloses which are rich in carbon, is even less suitable as a balanced substrate for microbial growth than the algal detritus in aquatic systems (Table 1).

Table 1. *Element ratios in three major components of forest ecosystems (Melillo & Gosz, 1983)*

	Carbon	Nitrogen	Phosphorus
Vegetation (woody tissue)	1500	10	1
Litter	500	10	1
Soil	120	9.4	1

In both soils and sediments the proportions of nitrogen and phosphorus relative to carbon increases in organic material after deposition. This is a result of the mineralisation of detrital organic carbon and the sequestration of nitrogen and phosphorus into the biomass of the microbial community which degrades the detritus. However, it appears from recent work that despite the C:N:P balance, it is only during the initial stages of detrital decay, when labile soluble carbon is present and available, that microbial degradation of detritus is nitrogen-limited. Thus, the rate of breakdown of wheat straw has been shown to be largely dependent upon the size of an immediately available soluble carbon pool, and of a pool of intermediately available carbon (Reinertson *et al.*, 1984). The addition of available carbon in the form of glucose to wheat straw stimulated its breakdown after 10 days' incubation, but not if added during the initial 10 days when microbial activity was nitrogen-limited (Knapp, Elliott & Campbell, 1983).

Deposited organic detritus is incorporated into the surface layers, the rate of mixing being strongly influenced by the activity of animals

which turn these over. O'Brien (1984) has followed downward movement of organic matter in soil by measuring variations in radiocarbon enrichment of organic matter with depth. In pasture soils, earthworms enhanced organic carbon incorporation and transport, in contrast to forest soils with a similar organic content where the smaller animals transported almost no organic matter downwards. Billen (1982) has also shown that bioturbation can influence significantly the vertical transport of organic matter and nutrients in surface layers of sediments.

The organic content of a soil or sediment may be very small in coarse-grained sands or gravels or very large in entirely organic soils. At any one time, it will be the result of a balance between the input of detritus and its removal by mineralisation. Thus, the organic content may be low because the input is low or because, despite a high input, it is being degraded rapidly. In the former case, flux of carbon and energy is small, in the latter case it is large. Much of the organic material may be refractory and unavailable to the microbiota, resulting in the accumulation of undegraded organics, as in the case of peats. Therefore, it is the turnover of the organic matter which determines the total flux of energy through the system, while it is the concentration of *available* organic carbon which determines the amount of energy available to the microflora at any one time.

Energy limitation of growth

Evidence suggests that in the overwhelming majority of soils and sediments the microflora is energy-limited, notwithstanding the large amounts of organic carbon which are sometimes present. Pamatmat *et al.* (1981) suggested that heat outputs from sediments were commensurate with the microbial community being energy-limited and that addition of available electron donors almost invariably stimulated respiration by the soil microflora. Addition of glucose to soil has been shown to stimulate respiration by the soil microflora, to increase soil microbial biomass and soil ATP (Nannipieri, Johnson & Paul, 1978; Sparling, Ord & Vaughan, 1981; Ahmed, Oades & Ladd, 1982) and to stimulate mineralisation of plant detritus (Knapp, Elliot & Campbell, 1983; Reinertsen *et al.*, 1984). Indeed, saturation of the microflora in soil by added glucose permits estimation of microbial biomass, since the maximum initial rate of glucose oxidation is proportional to the biomass present (Anderson & Domsch, 1978).

Following glucose addition, microbial biomass and activity increase but these revert to the original levels as glucose is depleted. Again, addition of hydrogen to slurries of marine sediment stimulates both sulphate reduction and methane formation, reversing the normal inhibition of methane formation by sulphate-reducing bacteria which outcompete methanogenic bacteria for electron donors (Abram & Nedwell, 1978); sulphate reduction in marine sediments is electron donor-limited (Nedwell & Abram, 1979).

The adenylate energy charge is a relative measure of the proportion of energised adenylate, in the form of ATP, compared to the lower-energy ADP and AMP. In metabolically active prokaryotic cells, the energy charge seems to be relatively high (between 0.8 and 0.9), reflecting rapid energy input into the cellular adenylates. In dormant or inactive cells the energy charge falls to values between 0.5 and 0.8, while at energy charges below 0.5 prokaryotic cells die. Eukaryotic cells may survive at these values, however. Energy charges between 0.3 and 0.4 have been measured for soil microorganisms. These charges increased after the addition of glucose but still to a value lower than that obtained for actively growing laboratory cultures (Martens, 1985). These data support the idea of an energy-limited microbial community in soil. However, Brookes, Tate & Jenkinson (1983) found much higher values for soil organisms, as did Tateno (1985) who recorded values of 0.85, reducing to 0.46 when soil was dried. Tateno suggested that inactive soil organisms with low energy charges will decompose quickly, an explanation which seems reasonable judging from the rapidity with which cells in chloroform-fumigated soils are decomposed (Jenkinson, 1966, 1976). Deaney & Gray (1983) have also shown that proteins, nucleic acids and antigens in stained cells decompose quickly in soil. There do not appear to be any measurements of energy charge in aquatic sediments, although their energy-limited nature can be deduced from the stimulation of microbial activity when electron donors are added.

A consequence of the limited energy available in soils and sediments is that growth rates and microbial production rates are slow. Calculations of approximate microbial growth rates, based on rates of carbon production compared to biomass carbon in the microbial standing crop, suggest slow growth rates with generation times of about 3.3 days in forest soil (Chapman & Gray, 1986) and about 2.6 days in marine sediments from the Kiel Fjord and Bight (Meyer-Reil *et al.*, 1980). However, estimates of generation times are very

Table 2. *Estimates of generation times of microorganisms in soils and sediments based on productivity:biomass ratios (PB); thymidine incorporation (THY); incorporation of adenine (ADEN); or turnover of ATP pool. Estimates based upon frequency of dividing cells have been omitted as the method over-estimates rates in sediments (Fallon, Newell and Hopkinson, 1983)*

Environment	Generation time (h)	Method	Reference
Soils			
Tundra	93	PB	Parinkina, 1974
Temperate	26–67	PB	Parinkina, 1974
Peat	39	PB	Clarholm & Rosswall, 1980
Humus	66	PB	Clarholm & Rosswall, 1980
Mineral soil	55	PB	Clarholm & Rosswall, 1980
Clay soil	3024	PB	Jenkinson & Ladd, 1981
Broadbalk	15 168	PB	Jenkinson & Ladd, 1981
Deciduous woodland	79	PB	Chapman & Gray, 1986
Fungi in soil	161–936	PB	Kjøller & Struwe, 1982
Sediments			
Sands, Kiel Fjord	63	PB	Meyer-Reil *et al.*, 1980
Zostera bed	3.8–125	THY	Moriarty & Pollard, 1981
Zostera bed	144–1008	THY	Moriarty & Pollard, 1982
Coastal muds	118–3049	THY	Fallon *et al.*, 1983
Six marine sites	4–166	ADEN	Craven & Karl, 1984
	212–350	ATP	Craven & Karl, 1984

variable and are very dependent upon the method used and the assumptions made (Chapman & Gray, 1986). A selection of data which illustrate this point is given in Table 2. Very long doubling times are obtained if biomass estimates are based upon direct microscopic or fumigation techniques. Indeed, some of the doubling times are so long that it is difficult to believe that they have any biological meaning. Nevertheless, whatever method is used, the generation times are long compared with those encountered in laboratory cultures. Part of the explanation for these extended generation times must also be that a large proportion of the microbial community in most natural environments is inactive (Gray & Williams, 1971*b*; Stevenson, 1978). The concept that only a small part of the community is active at any one time has attracted increasing support following the development of a number of techniques for *in situ* differentiation of active and inactive biomass and direct measurements of growth rates, e.g. autoradiography (Meyer-Reil, 1978),

Table 3. *Percentage of the viable biomass thought to be active in various natural environments*

Organisms	Habitat	Technique	% active	Reference
Bacteria	Forest soil			
	Al horizon	FDA	34	Lundgren & Söderström, 1983
	A2 horizon		54	
	B horizon		52	
	Agricultural			
	Barley field	ETS	15	Macdonald, 1980
	Manured soil		25	
	Turfed soil		11	
	Compost		23	
	Vegetable soil		23	
	Field soil		31	
	Aquatic			
	Water over sand	Tritiated glucose	2.3–56.2 average 31.3	Meyer-Reil, 1978
Fungi	Pine forest			
	A1 horizon	FDA	2.4	Söderström, 1979
	A2 horizon		4.3	
	B horizon		2.6	

FDA = hydrolysis of fluorescein diacetate
ETS = electron transport system activity

incorporation of [^{14}C]-thymidine (Fuhrman & Azam, 1982), the hydrolysis of fluoroscein diacetate (Söderström, 1979) and the reduction of dyes such as iodonitrotetrazolium by respiration (Iturriaga & Rheinheimer, 1975; Macdonald, 1980). Table 3 shows representative data from these investigations.

Soils or aquatic sediments therefore represent environments in which microorganisms, despite the possible presence of large amounts of organic matter, are energy- or nutrient-limited. A large proportion of recalcitrant organic matter and even some potentially labile material is not immediately available. A number of studies have shown that in marine sediments the chemical analysis of *in situ* concentrations of potential microbial substrates such as amino acids and short-chain fatty acids do not reflect the amounts available to bacteria (Christensen & Blackburn, 1980, 1982; Balba & Nedwell, 1982; Parkes, Taylor & Jørck-Ramberg, 1984). Thus, less than 0.01% of the measured alanine (Christensen & Blackburn, 1980) and 14% of the measured acetate (Christensen & Blackburn, 1982)

in sediments from the Limfjord in Denmark were readily available to the microflora. This reduction in availability seems to be due to the complexation of substrate molecules with high-molecular-weight organic molecules or metals (Christensen & Blackburn, 1982; Madsen & Alexander, 1985; Thompson & Nedwell, 1985) which, as discussed earlier, may or may not be adsorbed on particle surfaces.

It must be concluded therefore that soils and sediments are generally energy-limited environments in which microbial species adapted to efficient uptake and utilisation of nutrients will be at an advantage. However, the soil and sediment environments are heterogeneous with respect to time in that an occasional input of organic material creates a temporarily large supply of organic substrates, and seasonal inputs of detritus have similar temporary effects. Thus populations of microbes may persist because they are constantly but minimally active at extremely low available nutrient concentrations, or because they grow rapidly during short periods of nutrient abundance and survive in an inactive state during the long intervening times. These two strategies were reflected in the use of the terms autochthonous and zymogenous by Winogradsky (1924) and the more recent suggestion that both oligotrophic and copiotrophic organisms can coexist in nature (Poindexter, 1981). It is not necessary to suppose that these strategies are adopted by different organisms, however, for facultative oligotrophs are the organisms that might be best suited to a fluctuating environment. Thus, some copiotrophic bacteria respond to starvation by producing dwarf cells which have slower metabolic rates than the larger forms from which they are derived (Novitsky & Morita, 1977; Morita, 1982; Amy & Morita, 1983). Epifluorescence microscopy has been used to show that such cells comprise a significant proportion of the microflora of soils and sediments. Dow, Whittenbury & Carr (1983) have also shown that the swarmer cells of prosthecate bacteria have low metabolic rates and may thus represent an oligotrophic phase of an otherwise copiotrophic organism.

In a soil or sediment, a microbial cell will only be removed by predation or limited leaching, unlike the situation in a chemostat where the major loss of cells is usually due to wash-out. In a chemostat, a species with a greater affinity for a growth-rate-limiting nutrient may outcompete a second species which will then be removed from the system. In a soil or sediment, such rapid disappearance will not occur but the population will decline slowly as it attracts a diminishing proportion of the growth-limiting resource. Eventually, its

continued survival will depend upon its ability to meet its maintenance energy requirements and any mechanisms which enhance energy scavenging or reduce maintenance requirements will increase survival. Chapman & Gray (1981) have examined the maintenance energy requirements of the soil bacterium *Arthrobacter globiformis* which is able to survive starvation for many weeks in culture. They showed that as growth rate and temperature decreased, the amount of energy used for maintenance fell. Following starvation, the specific maintenance rate fell even further which could have been due to a shutting down of cell processes in the whole population, or part of the population of cells as previously described for *Escherichia coli* (Koch & Coffman, 1970). Chapman & Gray (1981) also showed that *A. globiformis* had a high true growth yield and under nitrogen-limiting conditions was capable of sequestering the carbon supply and converting it into larger quantities of glycogen which served as a reserve material.

Since dead cells are not washed out of the soil to any appreciable extent, it follows that they may in turn act as a source of energy for the growth of other cells, a phenomenon termed cryptic growth. Evidence from soils fumigated with chloroform demonstrates that dead microbial cells are quickly recycled, and Chapman & Gray (1986) have demonstrated that this could have an appreciable effect on microbial growth rates in soil. They cite an example where the average generation time of microorganisms in a deciduous woodland soil could be reduced from 8.3 days to 3.3 days. In this particular soil, they also showed that if the specific maintenance rate of the population rose above $0.006\,h^{-1}$, then all of the energy input would be used for maintenance, leaving none for growth. Unfortunately, values for the substrate inputs and microbial biomass in soil and the specific maintenance rate and true growth yield of the populations in these soils cannot be measured accurately and so calculations remain very imprecise.

It has also been suggested that energy and carbon limitation will promote adhesion of microbial cells to surfaces (Brown, Ellwood & Hunter, 1977) and it has been known for some time that organisms adsorbed onto surfaces such as glass beads can remain active at nutrient concentrations in the surrounding liquid which would not support growth in the absence of a surface (Jannasch & Pritchard, 1972). Both permanently and reversibly attached cells are better able to scavenge and metabolise organic molecules adsorbed on the surfaces of solid particles than are unattached cells (Kefford, Kjelleberg

& Marshall, 1982; Hermansson & Marshall, 1985) and thus adhesion may lead to the more rapid metabolism of substrates. Paerl & Merkel (1982) have shown that inorganic nutrients such as phosphate are also taken up more rapidly by bacteria attached to surfaces. Evidence that the metabolism of bacteria is increased at surfaces has also come from studies by Marshall, Stout & Mitchell (1971) who showed that bacteria adsorbed to glass were initially dwarf cells which reverted to normal sizes within 12–24 h. The ATP content per unit biovolume of three marine bacteria is known to be greater following attachment to glass (Kjelleberg & Dahlbeck, 1984) and uptake of labelled glucose and glutamate also increases (Kirschman & Mitchell, 1982). However, Jeffrey & Paul (1986) have shown that while the activity of attached cells of *Vibrio proteolytica* under energy-limiting conditions is greater than that of detached cells, the reverse is true under non-limiting conditions. In addition, attached cells are less sensitive to changes in nutrient concentration, their activity being independent of nutrient concentration below a critical threshold. The fact that attached cells might be buffered against changes in nutrient supply could be of even greater significance in soils and sediments where surfaces are so abundant.

REFERENCES

Abram, J. W. & Nedwell, D. B. (1978). Inhibition of methanogenesis by sulphate reducing bacteria competing for transferred hydrogen. *Archives for Microbiology*, **117**, 89–92.

Ahmed, M., Oades, J. M. & Ladd, J. N. (1982). Determination of ATP in soils: effect of soil treatments. *Soil Biology and Biochemistry*, **14**, 273–9.

Allison, F. E. (1968). Soil aggregation – some facts and fallacies as seen by a microbiologist. *Soil Science*, **106**, 136–43.

Amy, P S. & Morita, R. Y. (1983). Starvation-survival patterns of sixteen freshly isolated open-sea bacteria. *Applied and Environmental Microbiology*, **45**, 1109–15.

Anderson, J. P. E. & Domsch, K. H. (1978). A physiological method for the quantitative measurement of microbial biomass in soil. *Soil Biology and Biochemistry*, **10**, 215–21.

Balba, M. T. & Nedwell, D. B. (1982). Microbial metabolism of acetate, propionate and butyrate in anoxic sediments from the Colne Point salt marsh, Essex, UK. *Journal of General Microbiology*, **128**, 1415–22.

Billen, G. (1982). Modelling the processes of organic matter degradation and nutrient recycling in sedimentary systems. In *Sediment Microbiology*, ed. D. B. Nedwell & C. M. Brown, pp. 15–52. London, Academic Press.

Bosatta, E. & Agren, G. I. (1985). Theoretical analysis of decomposition of heterogeneous substrates. *Soil Biology and Biochemistry*, **17**, 601–10.

Bosatta, E. & Berendse, F. (1984). Energy or nutrient regulation of decomposition: implications for the mineralization–immobilization response to perturbations. *Soil Biology and Biochemistry*, **16**, 63–7.

BOSATTA, E. & STAAF, H. (1982). The control of nitrogen turnover in forest litter. *Oikos*, 39, 143–51.

BROOKES, P. C., TATE, K. R. & JENKINSON, D. S. (1983). The adenylate energy change of the soil microbial biomass. *Soil Biology and Biochemistry*, **15**, 9–16.

BROWN, C. M., ELLWOOD, D. C. & HUNTER, J. R. (1977). Growth of bacteria at surfaces: influence of nutrient concentration. *FEMS Microbiology Letters*, **1**, 163–6.

BURNS, R. G. (1983). Extracellular enzyme–substrate interactions in soil. In *Microbes in their Natural Environments*, ed. J. H. Slater, R. Whittenbury & J. W. T. Wimpenny, pp. 249–98. Cambridge, Cambridge University Press.

CHAPMAN, S. J. & GRAY, T. R. G. (1981). Endogenous metabolism and macromolecular composition of *Arthrobacter globiformis*. *Soil Biology and Biochemistry*, **13**, 11–18.

CHAPMAN, S. J. & GRAY, T. R. G. (1986). Importance of cryptic growth, yield factors and maintenance energy in models of microbial growth in soil. *Soil Biology and Biochemistry*, **18**, 1–4.

CHARD, J. M., GRAY, T. R. G. & FRANKLAND, J. C. (1983). Antigenicity of *Mycena galopus*. *Transactions of the British Mycological Society*, **81**, 503–11.

CHARD, J. M., GRAY, T. R. G. & FRANKLAND, J. C. (1985*a*). Purification of an antigen characteristic of *Mycena galopus*. *Transactions of the British Mycological Society*, **84**, 235–41.

CHARD, J. M., GRAY, T. R. G. & FRANKLAND, J. C. (1985*b*). Use of an anti-*Mycena galopus* serum as an immuofluorescence agent. *Transactions of the British Mycological Society*, **84**, 243–9.

CHRISTENSEN, D. & BLACKBURN, T. H. (1980). Turnover of tracer (^{14}C, ^{3}H-labelled) alanine in inshore marine sediments. *Marine Biology*, **58**, 97–103.

CHRISTENSEN, D. & BLACKBURN, T. H. (1982). Turnover of ^{14}C-labelled acetate in marine sediments. *Marine Biology*, **71**, 113–19.

CHUANG, T. Y. & KO, W. H. (1983). Propagule size: its relation to longevity and reproductive capacity. *Soil Biology and Biochemistry*, **15**, 269–74.

CLARHOLM, M. & ROSSWALL, T. (1980). Biomass and turnover of bacteria in a forest soil and peat. *Soil Biology and Biochemistry*, **12**, 49–57.

COOKE, R. C. & RAYNER, A. D. M. (1984). *Ecology of Saprotrophic Fungi*. London, Longman.

CRAVEN, D. B. & KARL, D. M. (1984). Microbial RNA and DNA synthesis in marine sediments. *Marine Biology*, **83**, 129–39.

DARBYSHIRE, J. F., ROBERTSON, L. & MACKIE, L. A. (1985). A comparison of two methods of estimating the soil pore network available to protozoa. *Soil Biology and Biochemistry*, **17**, 619–24.

DASHMAN, T. & STOTZKY, G. (1986). Microbial utilization of amino acids and a peptide bound on homoionic montmorillonite and kaolinite. *Soil Biology and Biochemistry*, **18**, 5–14.

DEANEY, N. B. & GRAY, T. R. G. (1983). Use of fluorochromes to determine viability of soil biomass. *Abstracts, Third International Symposium on Microbial Ecology*, 1983, 63.

DIX, N. J. (1984). Minimum water potentials for the growth of some litter decomposing agarics and other basiodiomycetes. *Transactions of the British Mycological Society*, **83**, 152–3.

DOW, C. S., WHITTENBURY, R. & CARR, N. G. (1983). The 'shut down' or 'growth precursor' cell – an adaptation for survival in a potentially hostile environment. In *Microbes in their Natural Environments*, ed. J. H. Slater, R. Whittenbury and J. W. T. Wimpenny, pp. 187–247. Cambridge, Cambridge University Press.

DOWSON, C. G., RAYNER, A. D. M. & BODDY, L. (1986). Outgrowth patterns of

mycelial cord-forming basidiomycetes from and between woody resource units in soil. *Journal of General Microbiology*, **132**, 203–11.

FALLON, R. D., NEWELL, S. Y. & HOPKINSON, C. S. (1983). Bacterial production in marine sediments: will cell-specific measures agree with whole system metabolism? *Marine Ecology Progress Series*, **11**, 117–19.

FITZPATRICK, E. A. (1971). *Pedology: A Systematic Approach to Soil Science*. Edinburgh, Oliver and Boyd.

FLETCHER, M. & MARSHALL, K. C. (1982). Are solid surfaces of ecological significance to aquatic bacteria? *Advances in Microbial Ecology*, **6**, 199–236.

FOSTER, R. C. & ROVIRA, A. D. (1976). Ultrastructure of the wheat rhizosphere. *New Phytologist*, **76**, 343–52.

FOTH, H. D. (1984). *Fundamentals of Soil Science*, 7th edn. New York, Wiley.

FRADKIN, A. & PATRICK, Z. A. (1982). Fluorescence microscopy to study colonization of conidia and hyphae of *Cochliobolus sativus* by soil microorganisms. *Soil Biology and Biochemistry*, **14**, 543–8.

FRANKLAND, J. C. (1984). Autecology and the mycelium of a woodland litter decomposer. In *The Ecology and Physiology of the Fungal Mycelium*, ed. D. H. Jennings & A. D. M. Rayner, pp. 241–60. London, Cambridge University Press.

FRANKLAND, J. C., BAILEY, A. D., HOLLAND, A. A. & GRAY, T. R. G. (1981). Development of an immunological technique for estimating mycelial biomass of *Mycena galopus* in leaf litter. *Soil Biology and Biochemistry*, **13**, 87–92.

FUHRMAN, J. A. & AZAM, F. (1982). Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. *Marine Biology*, **66**, 109–20.

GRAY, T. R. G., HISSETT, R. & DUXBURY, T. (1974). Bacterial populations of litter and soil in a deciduous woodland, II. – Numbers, biomass and growth rates. *Revue d'Ecologie et Biologie du Sol*, **10**, 15–26.

GRAY, T. R. G. & WILLIAMS, S. T. (1971*a*). *Soil Microorganisms*. London, Longman.

GRAY, T. R. G. & WILLIAMS, S. T. (1971*b*). Microbial productivity in soil. In *Microbes and Biological Productivity*, ed. D. E. Hughes and A. H. Rose, pp. 255–86. Cambridge, Cambridge University Press.

GREENLAND, D. J. (1971). Interactions between humic and fulvic acids and clays. *Soil Science*, **111**, 34.

GREENWOOD, D. J. (1961). The effect of oxygen concentration on the decomposition of organic materials in soil. *Plant and Soil*, **14**, 360–76.

GRIFFIN, D. M. (1972). *Ecology of Soil Fungi*. London, Chapman & Hall.

GRIFFIN, D. M. & LUARD, E. J. (1979). Water stress and microbial ecology. In *Strategies of Microbial Life in Extreme Environments*, ed. M. Shilo, pp. 49–63. Weinheim, Verlag Chemie.

HERMANSSON, M. & MARSHALL, K. C. (1985). Utilization of surface-localized substrate by non-adhesive marine bacteria. *Microbial Ecology*, **11**, 91–105.

HOFMANN, J. V. (1917). Natural reproduction from seed stored in the forest floor. *Journal of Agricultural Research*, **11**, 1–26.

ITURRIAGA, R. & RHEINHEIMER, G. (1975). A simple method for counting bacteria with active electron transport systems in water and sediment samples. *Kieler Meeresforschungen Sonderheft*, **31**, 83–6.

JANNASCH, H. W. & PRITCHARD, P. H. (1972). The role of inert particulate matter in the activity of aquatic microorganisms. *Memoire Istitut Italiano di Idrobiologia*, **29**, Supplement, 289–303.

JEFFREY, W. D. & PAUL, J. H. (1986). Activity of an attached and free-living *Vibrio* sp. as measured by thymidine incorporation, *p*-iodonitrotetrazolium reduction, and ATP/DNA ratios. *Applied and Environmental Microbiology*, **51**, 150–6.

JENKINSON, D. S. (1966). Studies on the decomposition of plant material in soil. II. Partial sterilization of soil and the soil biomass. *Journal of Soil Science*, **17**, 280–302.

JENKINSON, D. S. (1976). The effects of biocidal treatments on metabolisms in soil – IV. The decomposition of fumigated organisms in soil. *Soil Biology and Biochemistry*, **8**, 203–8.

JENKINSON, D. S. & LADD, J. N. (1981). Microbial biomass in soil: measurement and turnover. In *Soil Biochemistry*, vol. 5, ed. E. A. Paul & J. N. Ladd, pp. 415–71. New York, Dekker.

JONES, J. G. & SIMON, B. M. (1980). Decomposition processes in the profundal region of Blelham Tarn and the Lund tubes. *Journal of Ecology*, **68**, 493–512.

JØRGENSEN, B. B. (1977). Bacterial sulphate reduction within reduced microniches of oxidized marine sediments. *Marine Biology*, **41**, 7–17.

JØRGENSEN, B. B. (1982). Mineralisation of organic matter in the sea bed – the role of sulphate reduction. *Nature (London)*, **296**, 643–5.

JØRGENSEN, B. B. (1983). Processes at the sediment–water interface. In *SCOPE 21. The Major Biogeochemical Cycles and their Interactions*, ed. B. Bolin & R. B. Cook, pp. 477–509. Chichester, John Wiley.

KEFFORD, B., KJELLEBERG, S. & MARSHALL, K. C. (1982). Bacterial scavenging: utilization of fatty acids localized at a solid–liquid interface. *Applied and Environmental Microbiology*, **133**, 257–60.

KIRSCHMAN, D. & MITCHELL, R. (1982). Contribution of particle-bound bacteria to total microheterotrophic activity in five ponds and two marshes. *Applied and Environmental Microbiology*, **43**, 200–9.

KJELLEBERG, S. & DAHLBECK, B. (1984). ATP level of a starving surface-bound, and a free-living marine *Vibrio* sp. *FEMS Microbiology Letters*, **24**, 93–6.

KJØLLER, A. & STRUWE, S. (1982). Microfungi in ecosystems: fungal occurrence and activity in litter and soil. *Oikos*, **39**, 389–422.

KNAPP, E. B., ELLIOTT, L. F. & CAMPBELL, G. S. (1983). Microbial respiration and growth during the decomposition of wheat straw. *Soil Biology and Biochemistry*, **15**, 319–23.

KOCH, A. L. & COFFMAN, R. L. (1970). Diffusion, permeation, or enzyme limitation: a probe for kinetics of enzyme induction. *Biotechnology and Bioengineering*, **12**, 651–77.

LANYI, J. K. (1979). Life at low water activities: group report. in *Strategies of Microbial Life in Extreme Environments*, ed. M. Shilo, pp. 125–35. Weinheim, Verlag Chemie.

LUNDGREN, B. (1984). Size classification of soil bacteria: effects on microscopically estimated biovolumes. *Soil Biology and Biochemistry*, **16**, 283–4.

LUNDGREN, B. & SÖDERSTRÖM, B. (1983). Bacterial numbers in a pine forest soil in relation to environmental factors. *Soil Biology and Biochemistry*, **15**, 625–30.

MCCLAUGHERTY, C. A., PASTOR, J., ABER, J. D. & MELILLO, J. M. (1985). Forest litter decomposition in relation to soil nitrogen dynamics and litter quality. *Ecology*, **66**, 266–75.

MACDONALD, R. M. (1980). Cytochemical demonstration of catabolism in soil microorganisms. *Soil Biology and Biochemistry*, **12**, 419–23.

MADSEN, E. L. & ALEXANDER, M. (1985). Effects of chemical speciation on the mineralization of organic compounds by microorganisms. *Applied and Environmental Microbiology*, **50**, 342–9.

MARSHALL, K. C. (1976). *Interfaces in Microbial Ecology*. Cambridge, Massachusetts, Harvard University Press.

MARSHALL, K. C., STOUT, R. & MITCHELL, R. (1971). Selective sorption of bacteria from seawater. *Canadian Journal of Microbiology*, **17**, 1413–16.

MARSHMAN, N. A. & MARSHALL, K. C. (1981*a*). Bacterial growth on proteins in the presence of clay minerals. *Soil Biology and Biochemistry*, **13**, 127–34.

MARSHMAN, N. A. & MARSHALL, K. C. (1981*b*). Some effects of montmorillonite on the growth of mixed microbial cultures. *Soil Biology and Biochemistry*, **13**, 135–41.

MARTENS, R. (1985). Estimation of the adenylate energy charge in unamended and amended agricultural soil. *Soil Biology and Biochemistry*, **17**, 765–72.

MELILLO, J. M. & Gosz, J. R. (1983). Interactions of biogeochemical cycles in forest ecosystems. In *SCOPE 21. The Major Biogeochemical Cycles and their Interactions*, ed. B. Bolin & R. B. Cook, pp. 177–222. Chichester, John Wiley.

MEYER-REIL, L. A. (1978). Autoradiography and epifluorescence microscopy combined for the determination of number and spectrum of actively metabolising bacteria in natural waters. *Applied and Environmental Microbiology*, **36**, 506–12.

MEYER-REIL, L. A., BOLTER, M., DAWSON, R., LIEBEZEIT, G., SZWERINSKI, H. & WOLTER, K. (1980). Interrelationships between microbiological and chemical parameters of sandy beach sediment, a summer aspect. *Applied and Environmental Microbiology*, **39**, 797–802.

MORIARTY, D. J. W. & POLLARD, P. C. (1981). DNA synthesis as a measure of bacterial productivity in seagrass sediments. *Marine Ecology Progress Series*, **5**, 151–6.

MORIARTY, D. J. W. & POLLARD, P. C. (1982). Diel variation of bacterial productivity in seagrass (*Zostera capricornia*) beds measured by the rate of thymidine incorporation into DNA. *Marine Biology*, **72**, 165–73.

MORITA, R. Y. (1982). Starvation-survival of heterotrophs in the marine environment. *Advances in Microbial Ecology*, **6**, 171–98.

MORTLAND, M. M. (1970). Clay–organic complexes and interactions. *Advances in Agronomy*, **22**, 75.

NANNIPIERI, P., CECCANTI, B., CERVELLI, S. & SEQUI, P. (1978). Stability and kinetic properties of humus–urease complexes. *Soil Biology and Biochemistry*, **10**, 143–7.

NANNIPIERI, P., JOHNSON, R. L. & PAUL, E. A. (1978). Criteria for measurement of microbial growth and activity in soil. *Soil Biology and Biochemistry*, **10**, 223–9.

NEDWELL, D. B. & ABRAM, J. W. (1979). Relative influence of temperature and electron donor and electron acceptor concentrations on bacterial sulphate reduction in saltmarsh sediment. *Microbial Ecology*, **5**, 67–72.

NEWELL, K. (1984). Interaction between two decomposer basidiomycetes and a collembolan under Sitka Spruce: grazing and its potential effects on fungal distribution and litter decomposition. *Soil Biology and Biochemistry*, **16**, 235–40.

NOVITSKY, J. A. & MORITA, R. Y. (1977). Survival of a psychrophilic marine vibrio under long term nutrient starvation. *Applied and Environmental Microbiology*, **33**, 635–41.

O'BRIEN B. J. (1984). Soil organic carbon fluxes and turnover rates estimated from radiocarbon enrichment. *Soil Biology and Biochemistry*, **16**, 115–20.

PAERL, H. W. & MERKEL, S. M. (1982). Differential phosphorus assimilation in attached versus unattached microorganisms. *Archives for Hydrobiology*, **93**, 125–34.

PAMATMAT, H. M., GROF, G., BENTSSON, W. & NOVAK, C. S. (1981). Heat production, ATP concentration and electron transport activity of marine sediments. *Marine Ecology Progress Series*, **4**, 135–43.

PARINKINA, O. H. (1974). Bacterial production in tundra soils. In *Soil Organisms and Decomposition in Tundra*, ed. A. J. Holding, O. W. Heal, S. F. Maclean, Jr and P. W. Flanagan, pp. 65–77. Stockholm, Tundra Biome Steering Committee.

PARKES, R. J., TAYLOR, J. & JØRCK-RAMBERG, D. (1984). Demonstration, using *Desulfobacter* sp., of two pools of acetate with different biological availabilities in marine pore water. *Marine Biology*, **83**, 271–6.

POINDEXTER, J. S. (1981). Oligotrophy: fast and famine existence. *Advances in Microbial Ecology*, **5**, 63–89.

REINERTSEN, S. A., ELLIOTT, L. F., COCHRAN, V. L. & CAMPBELL, G. S. (1984). Role of available carbon and nitrogen in determining the rate of wheat straw decomposition. *Soil Biology and Biochemistry*, **16**, 459–64.

ROGERS, H. J. (1979). Adhesion of microorganisms to surfaces: some general considerations of the role of the envelope. In *Adhesion of Microorganisms to Surfaces*, ed. D. C. Ellwood, J. Melling and P. Rutter, pp. 29–55. London, Academic Press.

SERBAN, A. & NISSENBAUM, A. (1986). Humic acid association with peroxidase and catalase. *Soil Biology and Biochemistry*, **18**, 41–4.

SIALA, A. & GRAY, T. R. G. (1974). Growth of *Bacillus subtilis* and spore germination in an acid forest soil observed by a fluorescent antibody technique. *Journal of General Microbiology*, **81**, 191–8.

SIALA, A., HILL, I. R. & GRAY, T. R. G. (1974). Populations of spore-forming bacteria in an acid forest soil, with special reference to *Bacillus subtilis*. *Journal of General Microbiology*, **81**, 183–90.

SÖDERSTRÖM, B. A. (1979). Seasonal fluctuations of active fungal biomass in horizons of a podsolised pine-forest soil. *Soil Biology and Biochemistry*, **11**, 149–54.

SPARLING, G. P., ORD, B. G. & VAUGHAN, D. (1981). Microbial biomass and activity in soils amended with glucose. *Soil Biology and Biochemistry*, **13**, 99–104.

SPOSITO, G. (1984). *The Surface Chemistry of Soils*. Oxford, Oxford University Press.

STEVENSON, F. J. (1982). *Humus Chemistry*. New York, Wiley.

STEVENSON, L. H. (1978). A case for dormancy in aquatic systems. *Microbial Ecology*, **4**, 127–33.

STILLINGER, F. H. (1980). Water revisited. *Science, New York*, **209**, 451.

SUESS, E. (1980). Particulate organic carbon flux in the oceans – surface productivity and oxygen utilization. *Nature (London)*, **288**, 260–3.

SUESS, E. & MÜLLER, P. J. (1980). Productivity, sedimentation rate and sedimentary organic matter in the oceans. In *Biogéochemie de la Matière Organique a l'Interface Eau–Sediment Marin*, ed. R. Daumas, pp. 17–26. Paris, CNRS.

SWIFT, M. J., HEAL, O . W. & ANDERSON, J. (1979). *Decomposition in Terrestrial Ecosystems. Studies in Ecology*, vol. 5. Oxford, Blackwell.

TATENO, M. (1985). Adenylate energy charge in glucose-amended soil. *Soil Biology and Biochemistry*, **17**, 387–8.

THENG, B. K. G. (1979). *Formation and Properties of Clay–Polymer Complexes*. Amsterdam, Elsevier.

THOMPSON, L. A. & NEDWELL, D. B. (1985). Existence of different pools of fatty acids in anaerobic model ecosystems and their availability to microbial metabolism. *FEMS Microbiology Ecology*, **31**, 141–6.

VIGNEAUX, M., DUMON, J. C., FAUGERES, J. C., GROUSSET, F., JOUANNEAU, J. M., LATOUCHE, C., POUTIERS, J. & PUJOL, C. (1980). Matières organiques et sedimentation en milieu marin. In *Biogéochemie de la Matière Organique a l'Interface Eau–Sediment Marin*, ed. R. Daumas, pp. 113–28. Paris, CNRS.

WINOGRADSKY, S. (1924). Sur la microflore autochthone de la terre arable. *Comptes Rendus Hebdomadaires des Séances de l'Academie de Sciences, Paris*, **178**, 1236–9.